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Antibodies to wheat germ agglutinin in coeliac disease

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SUMMARY

Serum IgG and IgA antibodies to wheat germ agglutinin (WGA) were measured by an enzyme-linked immunosorbent assay (ELISA) with *N*-acetyl-D-glucosamine in all incubation steps to inhibit sugar-specific binding. Patients with coeliac disease (CD) had significantly higher antibody levels to WGA than patients with other intestinal disorders or healthy controls. Similar results were obtained for antibodies to the gluten fraction glyc-gli. The WGA antibodies did apparently not cross-react with gluten antigens, but commercial gluten powder contained traces of WGA or a similar lectin. Our findings support the proposal that WGA may be involved in the pathogenesis of CD.

Keywords coeliac disease gluten wheat germ agglutinin lectins ELISA

INTRODUCTION

It is not known how gluten exerts its harmful effect on the small intestinal mucosa in coeliac disease (CD). One possibility is that gluten acts as a lectin and binds to surface carbohydrate moieties of intestinal epithelial cells (Weiser & Douglas, 1976). However, the lectin properties of gluten are controversial (Köttgen *et al.*, 1982; 1983; Concon, Newburg & Eades, 1983; Auricchio *et al.*, 1984; Colyer *et al.*, 1984). We have recently purified a lectin from the gluten fraction called glyc-gli, which Douglas (1976) reported to bind to intestinal cell membranes. This lectin had physicochemical properties similar to wheat germ agglutinin (WGA) (Kolberg & Sollid, 1985). The lectin activity of gluten, therefore, may be due to trace amounts of WGA. Increased intestinal permeability and morphological changes of jejunal mucosa similar to the CD lesion have been reported in rats exposed enterically to WGA (Lorenzsonn & Olsen, 1982; Sjölander, Magnusson & Latkovic, 1984). These observations along with our detection of a WGA-like agglutinin in gluten prompted us to examine the IgG and IgA antibody response to purified WGA, crude gluten, glyc-gli and β-lactoglobulin in patients with CD and other intestinal disorders.

MATERIALS AND METHODS

Serum samples. Sera from venous blood were stored at −20°C for periods up to 7 years until tested. Samples were obtained from three groups:

(a) *Untreated CD.* Thirteen adults (10 women & 3 men; median age 34 years; range 19–67 years) and seven children (4 girls & 3 boys; median age 6 years; range 1–7 years), whose jejunal histology

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showed subtotal villous atrophy were studied. In addition serum from a CD patient with selective IgA deficiency was included to test the specificity of the immunoassay.

(b) *Other intestinal disorders.* Of the 15 adults studied (8 women & 7 men; median age 32 years; range 14–72 years), nine had Crohn's disease involving the terminal ileum, two had diarrhoea due to bacterial overgrowth in the small intestine, two had intestinal allergy, one had intestinal lymphoma, and one had WDHA (watery diarrhoea, hypokalaemia and achlorhydria) syndrome.

(c) *Healthy controls.* These were hospital staff, blood donors and children in good health (with informed consent of their parents) who were age- and sex-matched to the CD patients.

Antigens. WGA and β-lactoglobulin were obtained from Sigma Chemical Company (St Louis Missouri, USA). Crude gluten was obtained from ICN Pharmaceuticals (Cleveland Ohio, USA). The gluten fraction glyc-gli was prepared as described by Douglas (1976). In brief, 50 g of wheat gluten was suspended in 1·5 litres of 0·1 M ethanoic acid in 2·0 M ethanol (pH adjusted to 6·5 with NaOH). After continuous stirring for 12 h at 22°C, the mixture was centrifuged at 500 ***g*** for 15 min and the supernatant fluid collected. This fluid was left on ice for 1 h after addition of 250 ml of trichloroacetic acid (2.2 M). The precipitate was harvested by centrifugation at 18 000 ***g*** for 1 h at 4°C and redissolved in ethanoic acid (0·1 M). After dialysis for 2 days against isotonic Tris-HCl buffer, pH 7·2, the soluble fraction was cleared by centrifugation at 5,000 ***g*** for 15 min.

Enzyme-linked immunosorbent assay (ELISA). Costar EIA microplates (no. 3590) were coated with antigens at the following concentrations: WGA, crude gluten and glyc-gli, 0·05 g/l; and β-lactoglobulin, 0·01 g/l. Human sera were tested at 1:400 dilution for IgG and IgA activity by a non-competitive, double antibody sandwich ELISA as described previously (Scott *et al.*, 1984). All human serum and antiserum dilutions contained 0·1 M *N*-acetyl-D-glucosamine (Sigma Chemical Co., St Louis, Missouri, USA) to prevent sugar-specific binding of WGA to glycoproteins.

Absorption of sera from CD patients with gluten antigen. Diluted sera (1:200) from three CD patients with high antibody activities to all test antigens were absorbed by stirring with gluten powder (1 g/l or 100 g/l) for 18 h at 4°C. The supernatant fluids were collected by centrifugation at 30,000 ***g*** for 15 min (4°C) and tested in ELISA at a final dilution of 1010:400.

Statistical methods. Differences between groups were evaluated by Wilcoxon's test for unpaired samples (two tailed).

RESULTS

WGA can bind to carbohydrate moieties of immunoglobulins. Our unmodified ELISA was therefore unsuited for measurements of serum antibodies to WGA. Its predominant specificity is for *N*-acetyl-D-glucosamine (Rice & Etzler, 1974), which abolished sugar-specific binding when added to all serum dilutions at 0·1 M (Fig. 1). Negative for IgA anti-WGA in an IgA-deficient CD patient attested to the specificity of our modified ELISA.

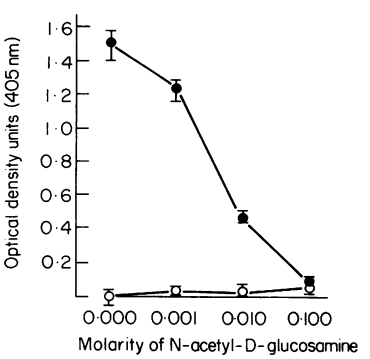

Fig. 1. Effect of various concentrations of *N*-acetyl-D-glucosamine in ELISA incubations on unwanted binding of reagents to coat (IgG) assay. Results show optical density readings (medians & observed range) with coat consisting of WGA (●) or gluten (○). All reagents except human serum included.

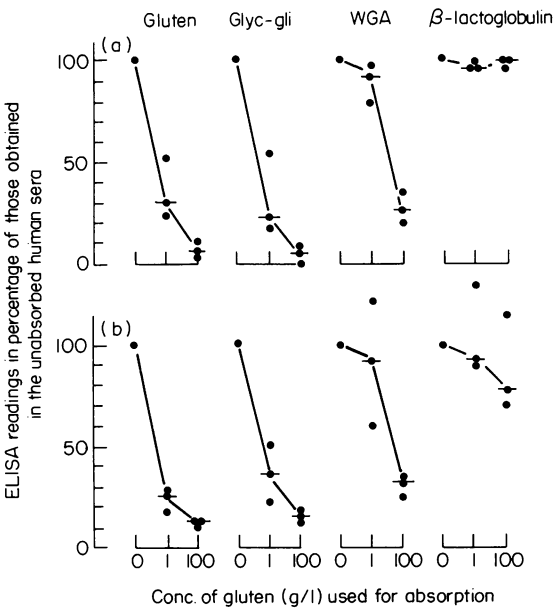

Fig. 2. IgG (a) and IgA (b) ELISA readings to crude gluten, glyc gli, WGA and β-lactoglobulin in three CD sera with raised antibody levels, tested unabsorbed and after absorption with crude gluten at 1 g/l or 100 g/l. Medians are indicated by horizontal lines.

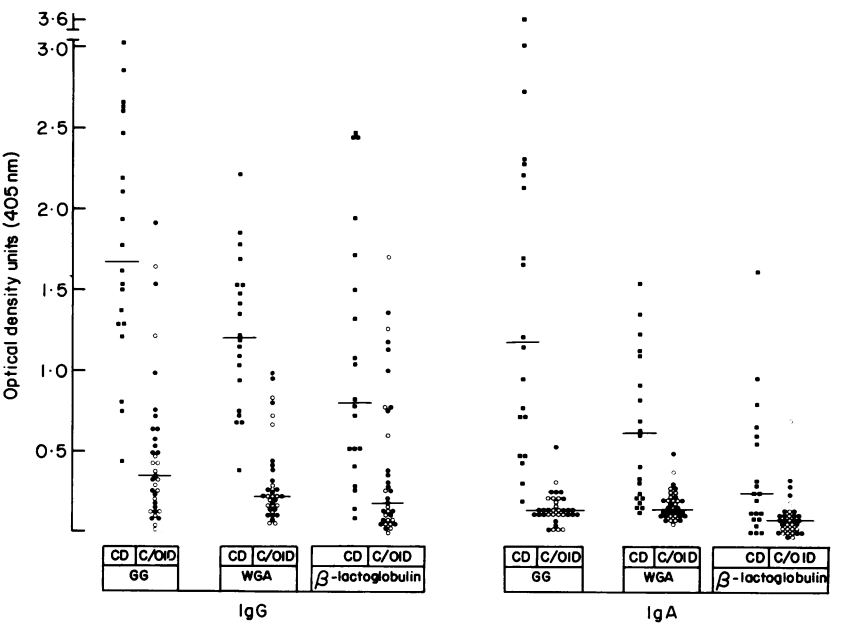

Fig. 3. Serum IgG and IgA antibody (ELISA readings to crude glyc-gli (GG), WGA and β-lactoglobulin in untreated CD patients (CD, ■), patients with other intestinal disorders (OID, ○), and healthy controls (C, ●). Medians are indicated by horizontal lines.

When CD sera (1:200) were absorbed with gluten (1 g/l), the ELISA readings to crude gluten antigen, and glyc-gli were diminished, while the activity to WGA was virtually unchanged. Absorptions with larger amounts of gluten (100 g/l) abolished activity to gluten and glyc-gli; the antibody levels to WGA were also considerably reduced whereas those to β-lactoglobulin were nearly unaffected (Fig. 2).

Untreated CD patients had higher IgG and IgA antibody levels ($P < 0{\cdot}001$) to glyc-gli, WGA and β-lactoglobulin than did healthy controls and patients with other intestinal disorders (Fig. 3). However, measurements of IgA and IgG antibodies to the wheat antigens appeared to discriminate much better between CD patients and other subject groups than did measurements of IgA and IgG antibodies to β-lactoglobulin (Fig. 3).

DISCUSSION

Using an ELISA in which all serum dilutions contained *N*-acetyl-D-glucosamine (0·1 M), to measure antibodies to WGA we observed that:

(a) Antibodies to WGA apparently did not cross-react with the main protein components of gluten.

(b) Commercial gluten powder contained trace amounts of an antigen that was similar to or identical with WGA: this finding has been further supported by our analyses of the lectin properties of the gluten fraction glyc-gli (Kolberg & Sollid, 1985).

(c) Antibodies to WGA were increased in sera from untreated CD patients similarly to antibodies to the gluten fraction glyc-gli.

Antibodies to wheat albumins and globulins are also raised in CD patients (Stern, Fisher & Gruttner, 1979; Kieffer *et al.*, 1982) and so are antibodies to dietary antigens other than wheat proteins (Kendrik & Walker Smith, 1970; Ferguson & Carswell, 1972; Burgin-Wolff *et al.*, 1976; Scott *et al.*, 1984). Both gluten and WGA antibodies, therefore, could solely signify altered intestinal permeability (Hamilton *et al.*, 1982; Bjarnason & Peters, 1983). However, the latter antibodies apparently discriminated CD patients from patients with other intestinal disorders and healthy controls better than antibodies to β-lactoglobulin. This finding may suggest that antibodies to WGA and gluten are particularly related to the pathogenesis of CD.

Lectins can bind to surface carbohydrate residues of cells, and some lectins have been reported to damage enterocytes of rats (King *et al.*, 1980; Wilson *et al.*, 1982; Lorenzsonn & Olsen, 1982; Sjölander *et al.*, 1984). Intraluminal administration of WGA produces dose-dependent lesions similar to CD (Lorenzsonn & Olsen, 1982), and permeability alterations (Sjölander *et al.*, 1984) characteristic of this disease (Chadwick, Phillips & Hofman, 1977; Walker & Bloch, 1983). The fact that WGA is resistant to gastrointestinal degradation (Brady, Vannier & Banwell, 1978) further strengthens its putative involvement in CD.

Some lectins are cytotoxic (Sharon & Lis, 1972), and others can mediate adherence of mouse macrophages to tumour cells. However, only WGA seems to induce killing of syngeneic and allogeneic tumour cells similarly to antibody-dependent cytotoxicity (Kurisu, Yamazaki & Mizuno, 1980; Tsunawaki *et al.*, 1983). WGA induced killing of uncoated erythrocytes by human cells with Fc receptors (Wei & Lindquist, 1983). In a human test system, WGA induces killing of uncoated erythrocytes by cells with Fc receptors (Wei & Lindquist, 1983). Binding of WGA to enterocytes and their lysis by Fc receptor-bearing effector cells may be one of the initial events in the development of CD. In this way WGA may induce an initial increase of intestinal permeability, thereby allowing enhanced uptake of dietary antigens including gluten. Hypersensitivity to a variety of dietary antigens may ensue.

WGA also inhibits mitogen- and antigen-induced proliferation of human blood mononuclear cells (Greene, Parker & Parker, 1976; Green & Waldmann, 1980), perhaps because it binds to and blocks the high-affinity Interleukin 2 (IL-2) receptors on T cells (Reed *et al.*, 1985), that are crucial for T-lymphocyte proliferation.

At low concentrations WGA induces limited proliferation of blood mononuclear cells (Gordon, Hamill & Parker, 1980; Green & Waldmann, 1980; Boldt & Dorsey, 1983), particularly the $CD8^+$

($T8^+$) subset (Boldt & Dorsey, 1983) which include suppressor and cytotoxic T cells. Their enhanced activity may provide an alternative explanation for inhibition excerted by WGA on mitogen- and antigen-induced lymphocyte proliferation (Reed *et al.*, 1985).

WGA may therefore affect the intestinal epithelial barrier and mucosal immune responsiveness in several ways. The raised antibody levels to WGA in CD patients suggests that its uptake may be increased, either as a primary or secondary event in the pathogenesis of this disease.

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